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NITROGEN AND PHOSPHORUS FORMS IN WATER AND THE FOOD REQUIREMENT OF ALGAE.

by Irena Spodniewska

(Wiadomosci Ekologiczne, 19, (3), pp 238-44.)

When dissolved in water, compounds of nitrogen and phosphorus ought to contain the basic assimilated food requirements for autotrophic plants and therefore autotrophic algae.

Nitrogen being a structural compound of protein cannot be replaced by any other element in the synthesis of this group of organic compounds. It is found in considerable amounts in organisms averaging about 3% of the total weight. Phosphorus, although occurring in lesser amounts than nitrogen, is likewise an essential nutrient since it participates in the building of many organic compounds. It occurs in numerous aliphatic substances (phosphatide), in nucleic acids, some proteins (phosphor protein) and hydrocarbons. Its presence is indispensable in many metabolic processes. Organic compounds of nitrogen and phosphorus undergo mineralisation during the decay of dead organic matter. In nature this continuous cycle of nitrogen and phosphorus and the ability to meet the nutrient demands of autotrophic plants, will for certain create itself the requirements for the existence of life. Supplies of organic material from living and dead organisms plus dissolved matter, biproducts of metabolism, are constant supplies of nitrogen and phosphorus compounds.

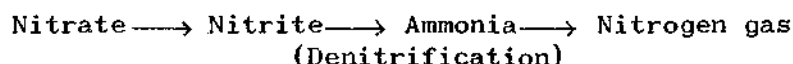
The concentration of various forms of nitrogen and phosphorus in water depends on the rate of mineralization of organic matter as well as the rate of uptake of nitrogen and phosphorus by plants. Likewise the amount of allochthonous matter flowing into the environment is critical. Being one of the major elements in air nitrogen also gets into water as molecular gas.

The break down processes of organic matter leading to the liberation of nitrogen are very complex and dependent to a considerable degree on the bacterial microflora. Under sterile conditions barely 20-30% of the nitrogen content in organic matter is liberated during autolysis of the cells in 3-5 days. A complete transfer of nitrogen from organic to mineral form comes about slowly. A gradual break down of protein occurs under the influence of proteolytic enzymes (peptides) derived through microorganisms. The end product of mineral protein containing nitrogen is ammonia. Thanks to the action of nitrifying bacteria the ammonia is oxidised to nitrite and thereafter to nitrate.

Ammonia → Nitrite → Nitrate
(Nitrification)

In areas of oxygen deficiency conditions are unfavourable for nitrification but on the other hand favour denitrification, causing

a reduction of nitrates, nitrites and ammonia; consequently releasing nitrogen gas.



Denitrification ceases in acidic waters (pH 6-7) but the activity of nitrifying bacteria is also limited and as a result nitrogen becomes a limiting factor for the growth of plants. A similar situation is met in strongly eutrophic environments, poor in oxygen. Considerable amounts of ammonia are found there as the only product in the break down of organic nitrogen.

More often different forms of nitrogen occur in water. In a great number of well oxidized and unpolluted waters the dominant form of nitrogen is nitrate. In general its concentration does not exceed 1mg.l^{-1} , average fluctuation lying between 0.1 - 0.5 mgsl^{-1} . In waters to which sewage is added the nitrate concentration rises to 5 - 10 mgsl^{-1} (sewage contains on the average about 80 mgsl^{-1} of nitrogen). Compounds of ammonia and nitrite occur in small quantities in pure waters. The concentration of nitrogen ammonia does not exceed 0.1 mgsl^{-1} in pure waters whereas in polluted water it can be 1mg.l^{-1} . Likewise nitrite is found in smaller quantities in pure water than in polluted water (respectively 0.01 and 0.05 mgsl^{-1}). Compounds of ammonia and nitrite are unstable and easily reduced therefore do not occur in large concentrations like nitrates.

Syrret (1962) collected and discussed the many data from experiments of different authors conducted with selected algal species in pure culture and assimilating particular forms of mineral nitrogen under various experimental conditions.

To the majority of autotrophic algae the usable forms of nitrogen are mineral compounds, primarily nitrate and ammonia compounds, if occurring in a suitable amount in the water. The growth rates of many algal species are similar, independent of whether they benefit from nitrate or ammonium salts, however, in low light intensities photosynthesis is generally faster in the presence of ammonium compounds. Often in the presence of nitrate and ammonium salts algae will utilize the latter preferentially and at times ammonia may even inhibit the uptake of nitrates. Likewise there are species of algae which do not utilize nitrates, notably Euglena gracilis Klebs. and Chlamydomonas reinhardtii Dang. Nitrates can be utilized by many species in small quantities but large concentrations of these salts have a detrimental effect on algae. Therefore nitrites can act as an indirect nitrogen source for autotrophic algae. Special interesting data include those showing a great physiological plasticity of algae. Algae cultured in a nitrogen deficient environment are characterized by a lesser nitrogen content in their dry weight; along with this is a change in the main products of photosynthesis. Species of Chlorella and Scenedesmus grown in a nitrogen deficient medium synthesize less protein and more carbohydrates and fats. Algae cultured first in nitrogen deficient conditions and then transferred to an environment high in mineral nitrogen show an ability to fix nitrogen in the dark up to a point where carbohydrates in the cells are beyond measure. A rise in the rate of respiration accompanies this process (4 - 5 times) and in some

species (eg. Navicula pelliculosa (Breb.) Hilse) also an assimilation of carbon dioxide in the dark. However, photosynthetic activity does not increase to the same degree as respiration.

Some species of algae have an ability to utilize organic nitrogen; Chlorella pyrenoidosa Chik. and Scenedesmus obliquus (Turp.) Kütz. thrive in the presence of urea. In mass cultures of Chlorella urea is used as the nitrogen source; it has a restrictive influence on bacterial growth and certainly produces a faster algal growth than mineral compounds. Certain blue green algae can also utilize urea. Chlorella vulgaris Beyerinck, Scenedesmus obliquus and species of Chlamydomonas can also take up amino acids (Syrret 1962).

Free nitrogen is inaccessible to the major part of algae, only a few blue greens manage like certain bacteria to utilize nitrogen gas (chiefly from the families Anabaena, Cylindrospermum and Nostoc). These organisms are of special importance to water ecosystems not abundant in mineral nitrogen compounds. The biochemical problem relating to molecular nitrogen fixation by blue-green algae was discussed by Fogg (1962). Like other nitrogen fixing organisms blue greens require molybdenum (0.1 mg l^{-1}), which is probably a special enzyme necessary in nitrogen fixation.

A considerable fluctuation in nitrogen concentration is met with in natural waters of differing type. In eutrophic lowland waters the amount of total nitrogen generally does not exceed 1 mg l^{-1} . A lot of the nitrogen is fixed in organic compounds, the amount of mineral nitrogen being small. In oligotrophic lakes nitrogen occurs mainly as mineral nitrates but in small quantities (Lityński 1952).

Within the surface layers of lakes there are considerable seasonal fluctuations in the nitrogen concentration. These are the consequence of seasonal changes in the growth activities of autotrophic organisms, changes in the rate of mineralization of organic matter as well as the availability of allochthonous matter. Many discrepancies arise in the concentration of nitrogen compounds within the different layers of water. The well illuminated surface layers are usually poor in nitrogen because it is taken up by plants, along with the sinking of decaying organisms. At the same time the amount of nitrogen increase in the deeper water layers where mineralization of organic matter takes place. Levelling out the concentration of nitrogen compounds throughout the water body involves the circulation of water and this will occur chiefly in deep reservoirs. In meromictic lakes where the surface and bottom layers are separated through a lack of circulation, a shortage of phytoplankton results due to nitrogen limitation. In the hypolimnium of meromictic lakes anaerobic bacteria reduce the nitrates to ammonia or even free nitrogen (Lityński 1952).

Now it is said that different algal species have a different nutrient requirement; not only concerning the form of nutrient they utilize but also the amounts which will guarantee optimum growth. According to Guseva (1952) diatoms have the least need for nitrogen, green algae the most with blue greens in between. Diatom requirement is 0.01 to 0.04 mg l^{-1} as nitrate nitrogen and green algae up to as much as 5 mg l^{-1} . Of course different species show optimum growth at different nitrogen levels; Dictyosphaerium demands a high nitrogen concentration at which the mass of algae in culture is proportional to the nitrogen concentration (even at $30\text{--}40 \text{ mg l}^{-1}$ of nitrate nitrogen). The optimum growth for

Oscillatoria agardhii Gomont is observed at a nitrate nitrogen concentration of 2 mgsl^{-1} and ammonia nitrogen of 0.2 mgsl^{-1} , for *Asterionella formosa* Hass. nitrate nitrogen is 0.2 mgsl^{-1} and ammonia nitrogen 0.02 mgsl^{-1} .

Increases in the mass of *Coelosphaerium* are not encountered at low nitrate concentrations ($4-10 \text{ mgsl}^{-1}$), instead growth is faster in the presence of ammonia nitrogen (optimum concentration $0.6-0.8 \text{ mgsl}^{-1}$). *Aphanizomenon flos aquae* (L.) Ralfs will grow better in the presence of ammonia nitrogen requiring in the region of 0.04 mgsl^{-1} , while species of *Anabaena* will grow better in the presence of nitrates (0.8 mgsl^{-1}).

From data quoted it appears that many species of algae require a high nitrogen concentration to attain optimal growth, such concentrations are not always present in nature. Usually an addition of mineral nitrogen to a lake will produce a rise in the primary production of the phytoplankton, an increase in algal mass and consequently an increase in fish production (Wróbel 1964, Francuzova, 1967, Włodek 1971).

From laboratory experiments with various species of algae the nitrogen-phosphorus relationship in an environment seems to have much influence on their growth. It is a well known fact that the ratio of nitrogen to phosphorus in different types of lakes is variable, according to Thomas (1964) it is 46 : 1 in an oligotrophic lake during winter, 11 : 1 in an eutrophic lake and 4 : 1 in a strongly eutrophic lake.

Data indicate that an enlarged phosphorus concentration enhances algal growth. However, the optimal phosphorus concentration for a given species may vary, depending upon the available form and the nitrogen concentration. In the presence of nitrates the optimal phosphorus requirement for algal growth is higher than in the presence of ammonium salts. In natural unpolluted waters the amount of phosphorus present is very low, between $0.000 - 0.140 \text{ mgsl}^{-1}$ (Hutchinson 1957). Repeatedly the observed spread in the number of polluted and eutrophic inland waters can be associated with a distinct rise in mineral substances, notably phosphorus.

According to Golachowska (1971), on work carried out in lakes of different trophic status in Poland (regarding depth differences), the total phosphorus in summer fluctuates between 0.022 mgsl^{-1} in a high oligotrophic mountain lake (Morskie Oko) to 4.6 mgsl^{-1} in the eutrophic lowland lake (Konarzyny). In lake Mikolajskie, data of author, the amounts of phosphorus at different depths in summer 1965 were $0.450 - 2.250 \text{ mgsl}^{-1}$, about 60% being organic phosphorus.

Phosphorus, both mineral and organic, is present in a dissolved form or as particulate in seston. According to Golachowska (1971) 40-60% of the total phosphorus in lakes is dissolved as orthophosphate 16-33% as dissolved organic phosphorus, 3-20% as organic phosphorus in seston and 4-12% as mineral phosphorus in seston. She emphasises however that the method of estimating the different fractions of phosphorus arouses reservations since it all passes from one form to another at a considerable rate. Changes which compounds of phosphorus undergo are dependent on the amounts of oxygen and iron

present in the water. In the presence of oxygen iron will form complexing substances with humic acid which have an ability to absorb phosphate ions. During summer stagnation in lakes, the lack of oxygen in the lower depths causes the passage of ferric into soluble compounds which consequently releases phosphorus. The formation of hydrogen sulphide will also release phosphorus (iron forms with difficulty soluble ferrous sulphide). In lakes oxygenated throughout ferrous phosphate is converted into soluble ferric phosphate, inducing the formation of a barrier which prohibits any further release of phosphorus from the lower depths. Oxygen deficiency during summer will enable phosphorus to pass from the bottom to the higher layers of water.

The wealth of a nutrient reserve depends not only on an absolute level of essential elements but also on the entire environmental requirements. Seasonal changes in phosphorus and nitrogen are similar; provided these are no seasonal changes in the inflow of allochthonous matter then the highest mineral phosphorus concentration will be evident during winter.

The release of phosphorus during the decay of organisms is an autolytic process resulting from enzymatic reactions without the aid of bacteria. Mineralization is therefore much quicker for phosphorus than nitrogen. During autolysis of cells of Scenedesmus obliquus nearly 50% of the total phosphorus was released in the course of a few hours under sterile conditions (Gotterman 1964). Processes of autolysis will stimulate further bacterial activity. The quick release of phosphorus during the break down of organic matter guarantees a residue of phosphorus in the surface water and enables its repeated utilization - the phosphorus concentration remains stable. Rigler (1961) paid attention to the role of ecoplankton in the break down of organic matter. The rate of mineralization of organic matter naturally depends on temperature. Despite a lower amount of phosphorus in the water during summer, its stability helps to satisfy the algal requirements.

Algae acquire phosphorus mainly as orthophosphate; only a few species benefit from organic phosphorus. The need of different species and groups of algae for phosphorus is variable. Rodhe (1948) singled out three groups of algae; the first develop in an environment of low phosphorous ($< 20 \text{ mg l}^{-1}$), to this belong Uroglena and Dinobryon; the second group is composed of algae with an optimum phosphorous concentration around 20 mg l^{-1} in natural conditions, here belong a great number of diatoms and blue greens. Green algae have the greatest phosphorus requirement, and dominate environments high in phosphorus ($> 20 \text{ mg l}^{-1}$), this is the third group having the greatest nutrient requirement.

Kuhl (1962) discussed at length problems connected with phosphorus uptake through algae, paying attention to a range of factors influencing the uptake rate and accumulation of phosphorus. It was discovered that light plays an important role in phosphorus uptake, which is greater in the light than dark. Likewise the phosphorus and hydrogen ion concentrations have much significance. The amount of phosphorus in the cells of many algae increases the phosphorus concentration in the environment, however, when there is a phosphate

excess the cell content will remain fixed. Gerloff and Skoog (1954) discussed a fourfold increase in the phosphorus content in cells of Microcystis aeruginosa Kütz at a high level of environmental phosphate.

Data quoted show at length the capacities of algae to adapt to environments of different nutrient wealth. This has unquestionable importance not only for the purpose of survival of a species but also in deciding indirectly about the stability of ecosystems.

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Notice

Please note that these translations were produced to assist the scientific staff of the FBA (Freshwater Biological Association) in their research. These translations were done by scientific staff with relevant language skills and not by professional translators.